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Expression analysis of MAC30 in human pancreatic cancer and tumors of the gastrointestinal tract

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Summmary. Meningioma-associated protein, MAC30, is a protein with unknown function and cellular localization that is differentially expressed in certain malignancies. In the present study, the expression of MAC30 in a variety of normal and cancerous human gastrointestinal tissues, with special emphasis on pancreatic tissues was analyzed. Quantitative RT-PCR was utilized to compare MAC30 expression levels. *In situ* hybridization and immunohistochemistry were carried out to localize MAC30 mRNA and protein expression in normal and cancerous tissue samples of the esophagus, stomach, colon and pancreas. Furthermore, the effects of TGF-ß on the transcription of MAC30 mRNA were examined in pancreatic cancer cells. MAC30 mRNA was expressed in a wide variety of normal human tissues, being most abundant in testicular and gastric tissue samples. MAC30 mRNA levels were significantly increased in breast and colon cancer, but significantly decreased in pancreatic and renal cancer. TGF-ß down-regulated MAC30 mRNA levels in certain pancreatic cancer cells. MAC30 protein was localized in normal pancreatic tissues, mainly in acinar and islet cells, and in normal colon, gastric and esophageal tissues especially in the mucosal cells. MAC30 was strongly present in tubular complexes in pancreatic cancer tissues but weak to absent in pancreatic cancer cells of primary tumors and metastases. In contrast, esophageal, gastric and colon tumors displayed strong MAC30 immunoreactivity in the cancer cells. In conclusion, MAC30 is expressed in various normal and diseased human tissues. MAC30 up-regulation in certain tumors and down-regulation in others suggests that this protein plays a distinct role in human malignancies.

Key words: MAC30, Pancreatic cancer, Chronic pancreatitis, Gastric cancer, Colon Cancer

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Introduction

Meningioma-associated protein (MAC30) is a recently identified protein with unknown function and cellular localization (Murphy et al., 1993). The *MAC30* gene is located on the long arm of chromosome 17 (17q11.2). It has a small segment of similarity to an apical gut membrane polyprotein of *Haemonchus contortus*, to olfactory receptor 30 of *Mus musculus*, and to cytochrome b in several organisms, yet there is no real sequence homology to any human gene. A wide variety of tissues, including brain, lung, heart, skeletal muscles, testis, ovary and pregnant uterus, express MAC30 mRNA (Malhotra et al., 1999).

MAC30 exhibits altered expression in certain human tumors. Thus it was first described to be overexpressed in meningiomas (Murphy et al., 1993, Malhotra et al., 1999), and using SAGE libraries, high levels of MAC30 mRNA are predicted in ovarian carcinoma, pancreatic adenocarcinoma, mammary adenocarcinoma, prostate carcinoma, colon adenocarcinoma, and brain medulloblastoma cell lines. MAC30 was found to be expressed as a non-erythropoietic gene in the fetal liver, yet it was not expressed in the adult liver, suggesting a possible role in growth and differentiation of this organ (Malhotra et al., 1999). The identification of MAC30 in the fetal liver was closely linked to the early hematopoietic period (10–12 weeks of gestation). This may suggest that it is one of the developmentally regulated proteins that are expressed only early in the hematopoietic development (Malhotra et al., 1999).

MAC30 expression is induced by other genes like *BRCA1* (Atalay et al., 2002) and mutations of this gene are of particular importance in ovarian and breast cancer as well as in other malignancies (Monteiro, 2003, Rosen et al., 2003). In addition, MAC30 can be down-regulated by the p53 tumor suppressor gene (Kannan et al., 2001). Also, MAC30 expression is repressed by JNK2 antisense in human PC3 prostate carcinoma cells (Potapova et al., 2002), after ectopic expression of telomerase in human mammary epithelial cells (HMECs) (Smith et al., 2003),

and after serum stimulation in fibroblasts (Iyer et al., 1999). In the current study we examined the expression of MAC30 mRNA in a number of normal and cancerous human tissues by quantitative RT-PCR. Moreover, we localized MAC30 protein in the normal and cancerous pancreas, chronic pancreatitis, and other malignant gastrointestinal tumors.

Materials and methods

Tissue sampling

Cancer tissue specimens were obtained from patients in whom tumor resection was performed. Normal human tissue samples were obtained through an organ donor program from previously healthy individuals. All samples were confirmed histologically. The organs included in this study and the corresponding numbers of cases are shown in tables 1 and 2. The age of the patients varied from 38 to 96 years (mean 66 years). Freshly removed tissues were fixed in paraformaldehyde solution for 12–24 h and then paraffin embedded for histological analysis. In addition, tissue samples were preserved in RNAlater (Ambion Europe Ltd., Huntingdon, Cambridgeshire, UK), and another portion was snap-frozen in liquid nitrogen immediately upon surgical removal and maintained at -80 °C until use. The Human Subjects Committees of the University of Bern, Switzerland, and the University of Heidelberg, Germany, approved all studies.

Table 1. Number of normal cases (n) for the indicated human tissues.

TISSUE	n
Adipose tissue	19
Adrenal gland	6
Bone marrow	1
Brain	6
Breast	3
Cervix	3
Colon	27
Esophagus	12
Heart	3
Hypothalamus	2
Kidney	4
Liver	7
Lung	22
Lymph node	4
Ovary	5
Pancreas	***32***
Placenta	3
Prostate	11
Rectum	1
Skeletal muscles	19
Skin	4
Small intestine	6
Spleen	5
Stomach	12
Testis	8
Thymus	1
Thyroid	1
Tonsil	2
Urinary bladder	8
Uterus	4

Antibody production

Mouse monoclonal antibodies (mAbs) for protein MAC30 were obtained by immunization with the synthetic peptide MKRKEKKNEGNNHWP representing the amino acid sequence (aa 1-15) deduced from the cDNA of MAC30 (NCBI, accession number XP 031536). As immunogen the peptide was used conjugated to keyhole limpet hemocyanin (KLH). For screening the carrier protein conjugated to the peptide was bovine serum albumin (BSA). Screening was performed using ELISA- and Western blot-techniques following standard protocols. The mAbs were raised essentially according to the method of Köhler and Milstein (Kohler and Milstein, 1975).

Cell culture

Pancreatic cancer cells were routinely grown in RPMI medium (Aspc-1, BxPc-3, Capan-1, Colo-357, and T3M4) or DMEM medium (Mia-PaCa-2 and Panc-1 cells), supplemented with 10% FBS and 100 U/ml penicillin (complete medium). For TGF-ß1 induction experiments (Guo et al., 2003, Kayed et al., 2003), cells were seeded in 24-well plates in 10% FBS growth medium and allowed to attach for 12 h. Growth medium was replaced by serum-reduced medium (0.5% FBS), supplemented with 200 pM TGF-ß1 (R&D Systems GmbH, Wiesbaden, Germany) for the indicated time periods. mRNA was isolated as described below.

Immunohistochemistry

Paraffin-embedded tissue sections 2-3 µm thick were immunostained using the DAKO Envision TM + system (DAKO Corporation, Carpinteria, CA, USA). Tissue sections were deparaffinized in xylene and rehydrated in progressively decreasing concentrations of ethanol. Thereafter, the slides were placed in washing buffer (10 mM Tris-HCl, 0.85% NaCl, 0.1% bovine serum albumin, pH 7.4) and subjected to immunostaining.

Table 2. Number of cancer cases (n) for the indicated human tissues.

TISSUE	n
Breast	7
Colon	40
Esophagus	5
Kidney	5
Lung	14
Pancreas	***54***
Prostate	18
Stomach	7
Urinary bladder	4

After antigens were retrieved by boiling the tissue sections in 10 mM citrate buffer for 10 min in the microwave oven, sections were incubated with normal goat serum (DAKO) for 45 min to block non-specific binding sites. Next, sections were incubated with a mouse monoclonal anti-MAC30 antibody diluted 1:5 at 4 °C overnight. The slides were then rinsed with washing buffer and incubated with anti-mouse-labeled polymer HRP (DAKO) for 45 min at room temperature. Tissue sections were then washed in washing buffer and each section was subjected to 100 µl DAB-chromogen substrate mixture (DAKO) and then counterstained with Mayer's hematoxylin. Sections were washed, dehydrated in progressively increasing concentrations of ethanol and mounted with Xylene-based mounting medium (Friess et al., 1993; Guo et al., 2003; Kayed et al., 2003). Slides were analyzed using the Axioplan 2 imaging microscope (Carl Zeiss light microscope, Göttingen, Germany).

In situ hybridization

Specific human MAC30 riboprobes were generated by RT-PCR using the following primer pairs: sense, 5´-ACAGACTATGGGGGCTCC-3´; antisense, 5´-GAATCCACTTGCAGCTTCCT-3´. The resulting PCR products were subcloned into the pGEM-T easy vector (Promega GmbH, Mannheim, Germany) containing promoters for DNA-dependent SP6 and T7 RNA polymerases. The authenticity of the subcloned 295-base-pair MAC30 fragment was confirmed by sequencing (Sequence Laboratories, Göttingen GmbH, Göttingen, Germany). Plasmids were linearized using SpeI and NcoI restriction enzymes. T7 and SP6 RNA polymerases were used to construct sense and antisense complementary RNA riboprobes. Biotin complementary RNA labeling was performed using the biotin RNA labeling kit according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany). Tissue sections (3 µm) were deparaffinized, rehydrated with 1x phosphate-buffered saline, and incubated in 0.2 mol/L HCl for 20 min at room temperature. After rinsing of the slides in 2x standard saline citrate, sections were treated with proteinase K (Roche) at a concentration of 25 µg/mL for 15 min at 37 °C. After postfixation with 4% paraformaldehyde in phosphate-buffered saline for 5 min and washing in 2x standard saline citrate, samples were acetylated in 2.5% acetic anhydride and 1.5% triethanolamine for 10 min. Subsequently, sections were prehybridized at 70 °C for 2 h in 50% formamide, 4x standard saline citrate, 2 x Denhardt's reagent, and 250 µg RNA/mL Hybridization was performed overnight at 70°C in 50% formamide, 4x standard saline citrate, 2x Denhardt's reagent, 500 µg RNA/mL, and 10% dextran sulfate. The final concentration of the biotin-labeled probes was 0.8 ng/µL. After hybridization, excess probe was removed by washing the slides three times in Dako stringent wash solution (DAKO) at 75 °C for 15 min. The samples were then incubated with streptavidin alkaline phosphatase conjugate (DAKO) for 30 min at room temperature. For color reaction, 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium substrate (DAKO) was used (Kleeff et al., 1998, Reubi et al., 2003).

Real-time quantitative polymerase chain reaction (QRT-PCR

All reagents and equipment for mRNA/cDNA preparation were supplied by Roche Applied Science (Mannheim, Germany). mRNA of human pancreatic tissues was prepared by automated isolation using the MagNA pure LC instrument and isolation kit I (for cells) and kit II (for tissues). cDNA was prepared using the first strand cDNA synthesis kit for RT-PCR (AMV) according to the manufacturer's instructions. Real-time PCR was performed using the LightCycler FastStart DNA SYBR Green kit. The number of specific transcripts (MAC30) was normalized to housekeeping genes (cyclophilin B and HPRT). The transcript number was calculated from a virtual standard curve, obtained by plotting a known input concentration of a plasmid to the PCR-cycle number at which the detected fluorescence intensity reaches a fixed value. The data of the two independent analyses for each sample and parameter were averaged and presented as adjusted transcripts/µl cDNA (Guo et al., 2003, Kayed et al., 2003).

For the other human tissue samples, total RNA was extracted using Ultraspec RNA isolation kits (Biotecx, BL10100), and further purified using RNeasy mini kits (Qiagen). Fifteen mg total RNA was converted into double-stranded cDNA by reverse transcription (GIBCO BRL Life Technologies, Grand Island, NY) using the T7-T24 primer (5'-GGC CAG TGA ATT GTA ATA CGA CTC ACT ATA GGG AGG CGG (dT24)) and cleaned up by Phenol/Chloroform/Isoamyl extraction using phase lock gel (5 Prime-3 Prime Inc.). Master 384-well plates were generated containing 5 ng/ml double-stranded cDNA. Daughter plates were produced (final cDNA concentration: 40 pg/ml (200 pg/well)) either manually or via robotics. Duplex Real-Time PCR (target gene and GAPDH as reference gene) on 384-well optical plates was performed using TaqMan® technology and analyzed on an ABI Prism® PE7900 Sequence Detection System (Perkin-Elmer Applied Biosystems (PE), Lincoln, CA), which uses the 5'nuclease activity of Taq DNA polymerase to generate a real-time quantitative DNA analysis assay. PCR mix per well (25 ml) consisted of commercially available, premixed GAPDH TaqMan® primers/probe (PE), 900nM each of 5' and 3' primers and 200nM TaqMan® probe from each target gene, 200pg cDNA and TaqMan® Universal PCR Master Mix (PE). The following PCR conditions were used: 50 °C for 2 minutes, then 95 °C for 10 minutes, followed by 40 cycles at 95 °C for 15 seconds and 62 °C for 1 minute. The expression levels of target genes were normalized to reference gene levels and represented as relative expression (E) where DCt is the difference between

reference and target gene cycles at which the amplification exceeds an arbitrary threshold. For the reference gene values, GAPDH Ct values were for each tissue were adjusted based on expression values of a panel of eight housekeeping genes, in order to further improve normalization.

Statistical analysis

Results are expressed as the mean ± standard error of the mean (SEM) unless indicated otherwise. For statistical analysis the Student's t test was used. Significance was defined as $p<0.05$.

Results

Previously, using DNA microarrays we identified *MAC30* as a differentially expressed gene in pancreatic cancer (Friess et al., 2003). To further quantify MAC30 mRNA expression we performed QRT-PCR in normal pancreatic tissues (n=19), pancreatic cancer (n=30), and chronic pancreatitis samples (n=22). MAC30 mRNA was expressed in normal pancreatic tissues (range 2000-6500 copies/µl) (Fig. 1A), with only one sample exhibiting significantly higher levels of MAC30 mRNA (15,000 copies/µl). 50% of pancreatic cancer tissues exhibited MAC30 mRNA expression below the range of MAC30 levels in normal pancreatic tissues, 30% exhibited MAC30 mRNA levels above the range, and 20% of pancreatic cancer cases exhibited MAC30 mRNA expression levels within the range in normal pancreatic tissues. In contrast, 32% of CP samples exhibited mRNA expression levels above the range in normal pancreatic tissues, 55% fell within the normal range, and 13% of cases expressed MAC30 mRNA below the range of MAC30 levels in normal pancreatic tissues.

Since pancreatic cancer tissues expressed variable levels of MAC30 mRNA, in the next set of experiments we analyzed the expression of MAC30 mRNA in pancreatic cancer cell lines by QRT-PCR. This analysis revealed that all pancreatic cancer cells expressed variable levels of MAC30 mRNA (Table 3). Colo-357 displayed the highest copy number of MAC30 mRNA, while the lowest MAC30 mRNA copy number was observed in Aspc-1 cells. Since TGF-ß1 is known to be overexpressed in pancreatic cancer (Friess et al., 1993), we examined the effect of TGF-ß1 on MAC30 mRNA expression levels in pancreatic cancer cells. MAC30 mRNA expression in Colo-357 and Panc-1 was significantly repressed by TGF-ß1, with a maximum of 60% and 35%, respectively (Figure 1B). In contrast, there were no significant effects of TGF-ß1 on MAC30 mRNA expression in the other pancreatic cancer cell lines.

In the next experiments we localized MAC30

Table 3. MAC30 mRNA expression in pancreatic cancer cell lines. mRNA input was normalized to the average expression of the two housekeeping genes HPRT and Cyclophilin B and is presented as copy number/µl cDNA.

CELL LINE	mRNA
Aspc-1	659
BxPC-3	1334
Capan-1	1206
Colo-357	5505
MiaPaCa-2	3618
Panc-1	2529
T3M4	2549

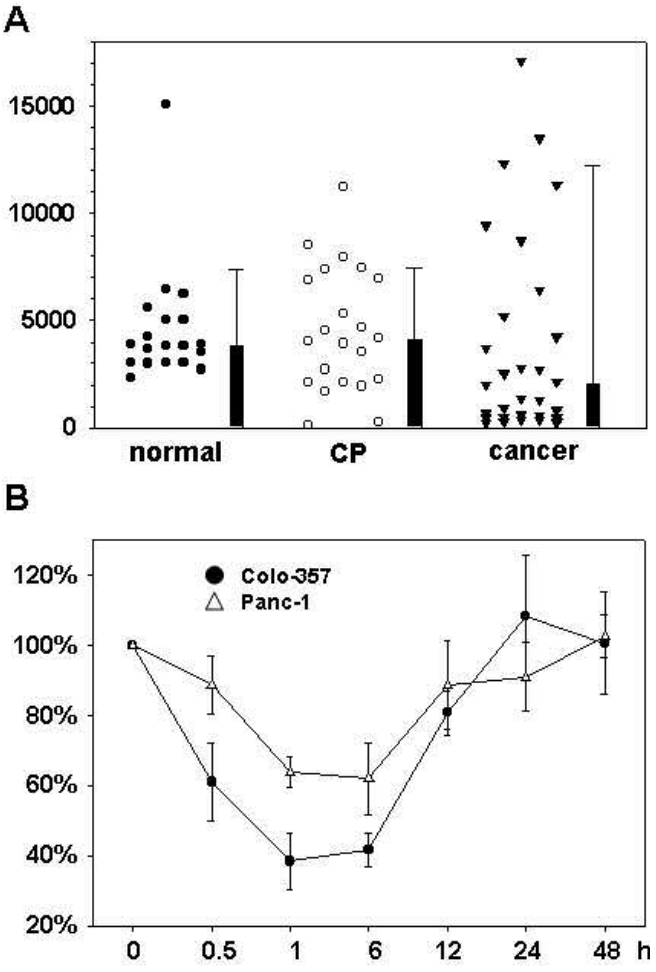

Fig. 1. QRT-PCR analysis of MAC30. RNA was extracted from normal pancreatic tissues (n=19), CP samples (n=22) and pancreatic cancer samples (n=30) as described in the Methods section. **A.** Depicts the variations in MAC30 mRNA expression in both CP and pancreatic cancer tissues compared to normal controls. RNA input was normalized to the average expression of the two housekeeping genes HPRT and cyclophilin B and is presented as mean copy number/µl cDNA ± SD. **B.** MAC30 mRNA regulation by TGF-ß1 in Colo-357 (black circle) and Panc-1 (white triangle) pancreatic cancer cells by QRT-PCR. Cells were incubated with 200 pM TGF-ß1 for the indicated time as described in the Methods section. Values represent the mean ± SEM of 3 separate experiments.

mRNA by *in situ* hybridization in normal (n=6) and cancerous (n=12) pancreatic tissue samples. In normal pancreatic tissues, MAC30 mRNA was expressed in the acini, islets and large ducts (Fig. 2A,B). MAC30 mRNA expression was very weak in pancreatic cancer cells (Fig. 2C). In contrast, tubular complexes in CP-like areas of pancreatic cancer samples exhibited strong expression of MAC30 mRNA (Fig. 2C,D). Because MAC30 mRNA was expressed in pancreatic tissues, we next studied the expression and localization of MAC30 protein in normal (n=10), CP (n=15), and pancreatic cancer tissues (n=21), and in pancreatic cancer metastases to the liver (n=3) and lymph nodes (n=2). In the normal pancreas, MAC30 was strongly expressed in the cytoplasm of pancreatic islet cells, specifically in the peripheral cells within the islets (Fig. 3C). MAC30 was weakly to moderately expressed in pancreatic acini but absent in the small-sized pancreatic ducts (Fig. 3A-C). MAC30 immunoreactivity was also present in the endothelial and smooth muscle cells of the blood vessels, as well as in nerves, monocytes, and fibroblasts. In both CP and pancreatic cancer tissues MAC30 was strongly expressed in tubular complexes (Fig. 3E,F). Moreover, dilated pancreatic ducts and duct cells forming PanIN lesions exhibited strong MAC30 immunostaining (Fig. 3D,G). In pancreatic cancer tissues, 75% of cases exhibited weak to absent MAC30 immunoreactivity in the cancer cells (Fig. 3 H). In contrast, 25% of pancreatic cancer cases exhibited moderate to strong MAC30 immunostaining (Fig. 3I,K,L). In addition, lymph nodes and liver metastases exhibited weak to absent cytoplasmic MAC30

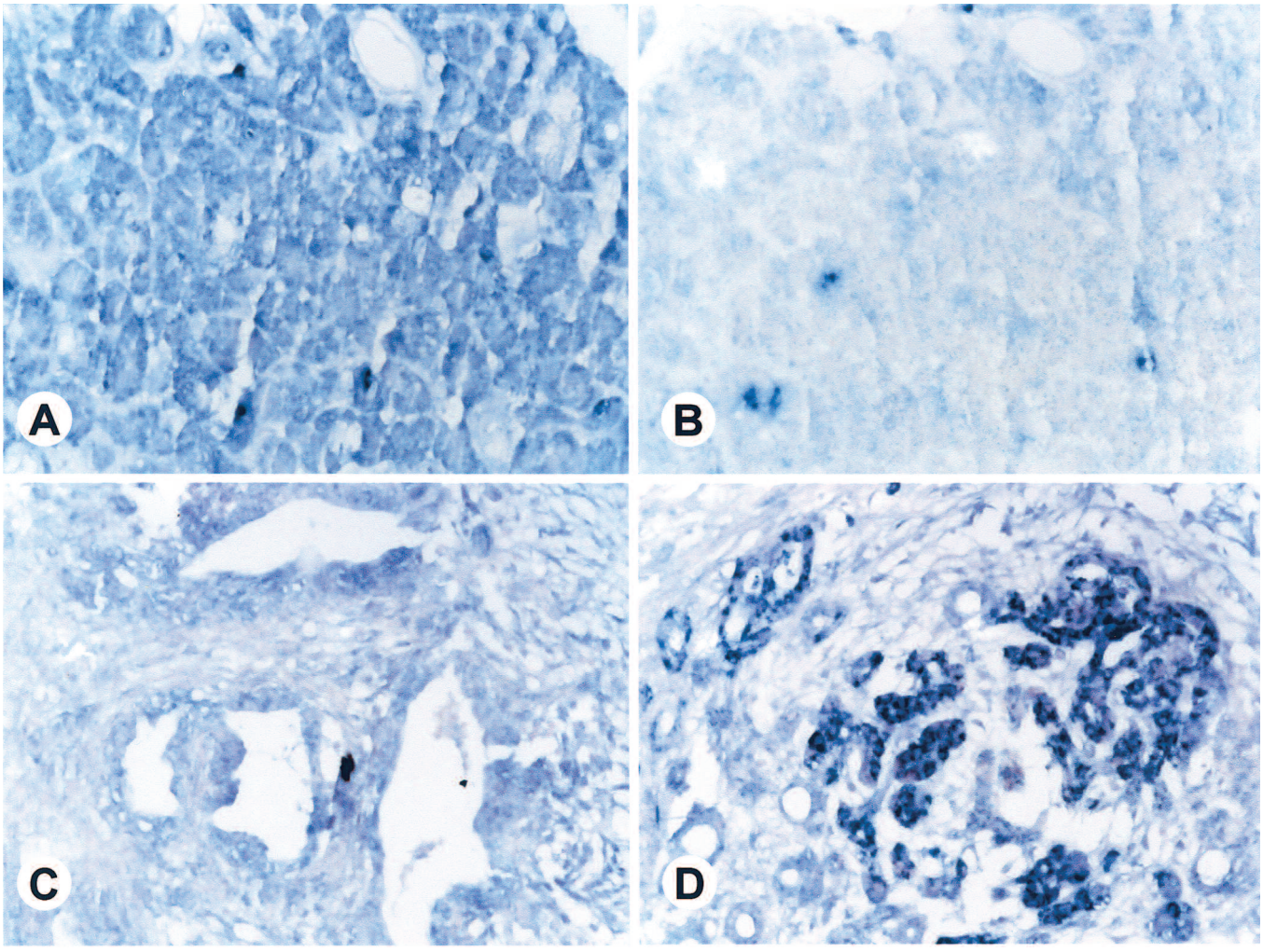

Fig. 2. MAC30 mRNA localization in pancreatic tissues. In situ hybridization was performed as described in the Methods section. Consecutive normal pancreatic tissue sections incubated with MAC30 antisense **(A)** and sense **(B)** probes showed specific acinar MAC30 mRNA expression. **C.** Pancreatic cancer tissues incubated with MAC30 antisense probe showed weak MAC30 mRNA expression in the cancer cells. **D.** Strong MAC30 mRNA expression in tubular complexes. Original magnification x 100

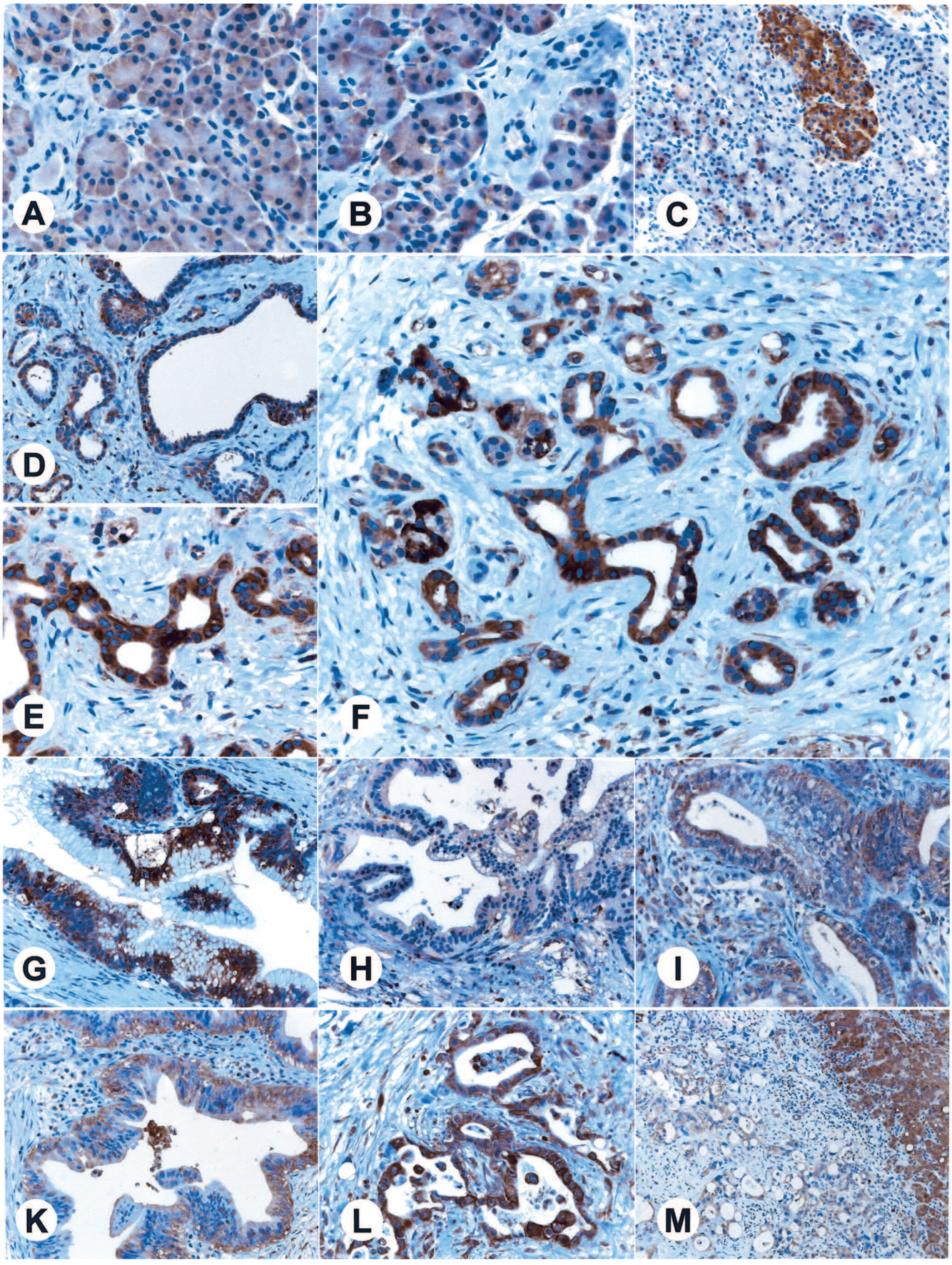

Fig. 3. MAC30 expression and localization in pancreatic tissues. Immunohistochemistry was performed as described in the Methods section. **A-C.** MAC30 in normal pancreatic ducts, acini **(A, B)** and islets **(C)**. **D.** Dilated ducts and small ducts in CP. **E and F.** Tubular complexes in CP-like lesions in pancreatic cancer. **G.** PanIN-1a formation in CP. Weak **(H)**, moderate **(I, K)** and strong **(L)** MAC30 immunostaining in pancreatic cancer cells. **M.** Liver metastases of pancreatic cancer; note the weak MAC30 staining in the cancer cells (left side). Original magnification: A, B, E-G, x 100; C, D, H, I, K, L, x 200; M, 20

immunostaining in the cancer cells (Fig. 3M).

Next, we screened a number of normal human tissues by QRT-PCR for the expression of MAC30. The highest expression of MAC30 mRNA was observed in testis and stomach tissues (Fig. 4A). Lower levels of MAC30 mRNA were observed in the other tested human tissues, which expressed MAC30 mRNA at variable levels. We compared MAC30 mRNA levels among normal and cancerous human tissues in six different organs by QRT-PCR. A significant increase in MAC30 mRNA levels of breast and colon cancer was observed in comparison to the normal controls (Fig. 4B). In contrast, a significant reduction of MAC30 mRNA expression levels was found in renal cancer compared to normal renal tissues. No significant difference in the expression of MAC30 mRNA levels in normal versus cancerous tissues of the urinary bladder, lung or prostate was observed. Immunohistochemistry was carried out in various normal (n=5) and cancerous (n=5) tissue samples, i.e., esophagus (3 adenocarcinomas and 2 squamous cell carcinomas), stomach and colon adenocarcinoma. In the normal esophagus, MAC30 was strongly expressed in the cytoplasm of mucosal cells. The superficial and intermediate epithelial layers exhibited strong MAC30 immunostaining, whereas the basal epithelial layer exhibited weak or absent MAC30

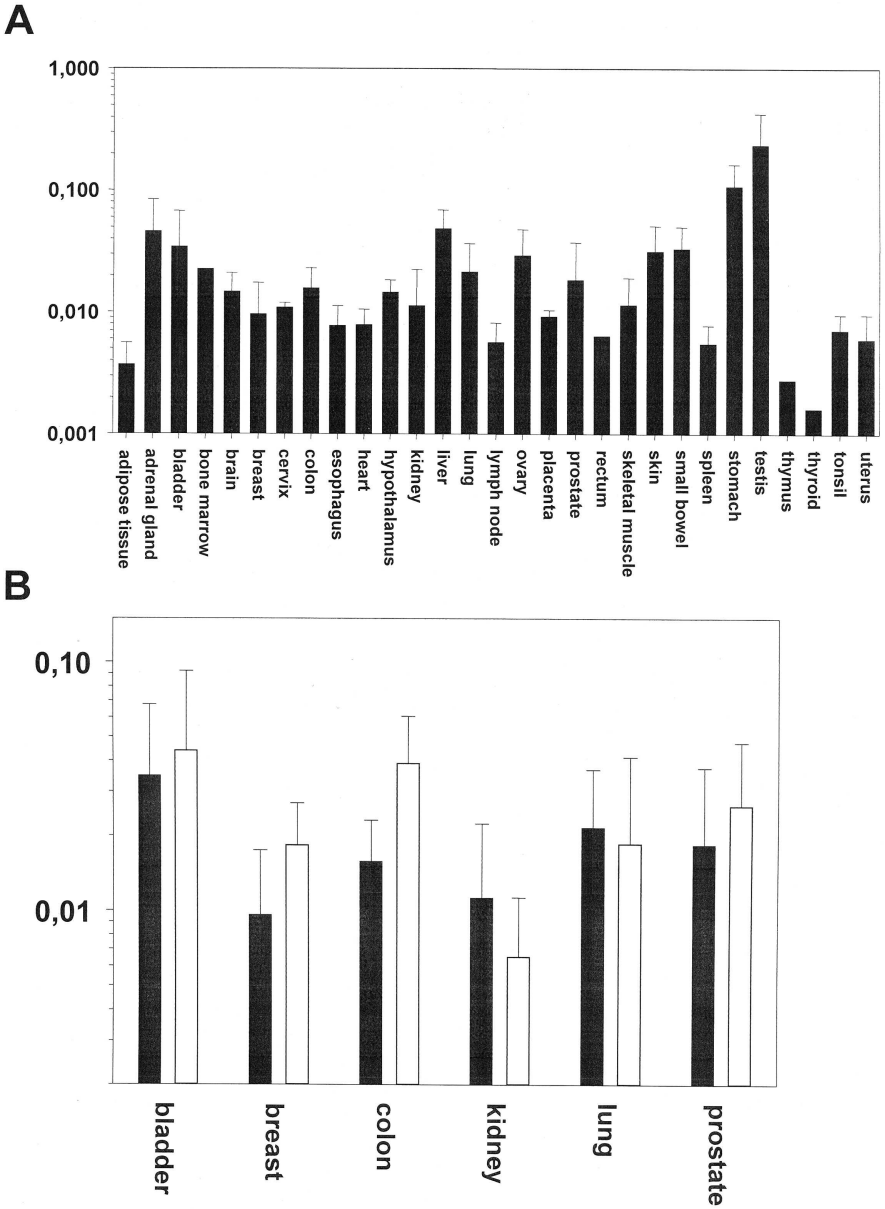

Fig. 4. MAC30 mRNA expression in human tissues by QRT-PCR. RNA was extracted and QRT-PCR was performed as described in the Methods section. **A.** MAC30 mRNA expression in different human tissues as indicated. **B.** Comparison between MAC30 mRNA expression in normal (black) and cancerous (white) tissues of different organs as indicated. Values represent arbitrary units normalized to housekeeping genes ± SD.

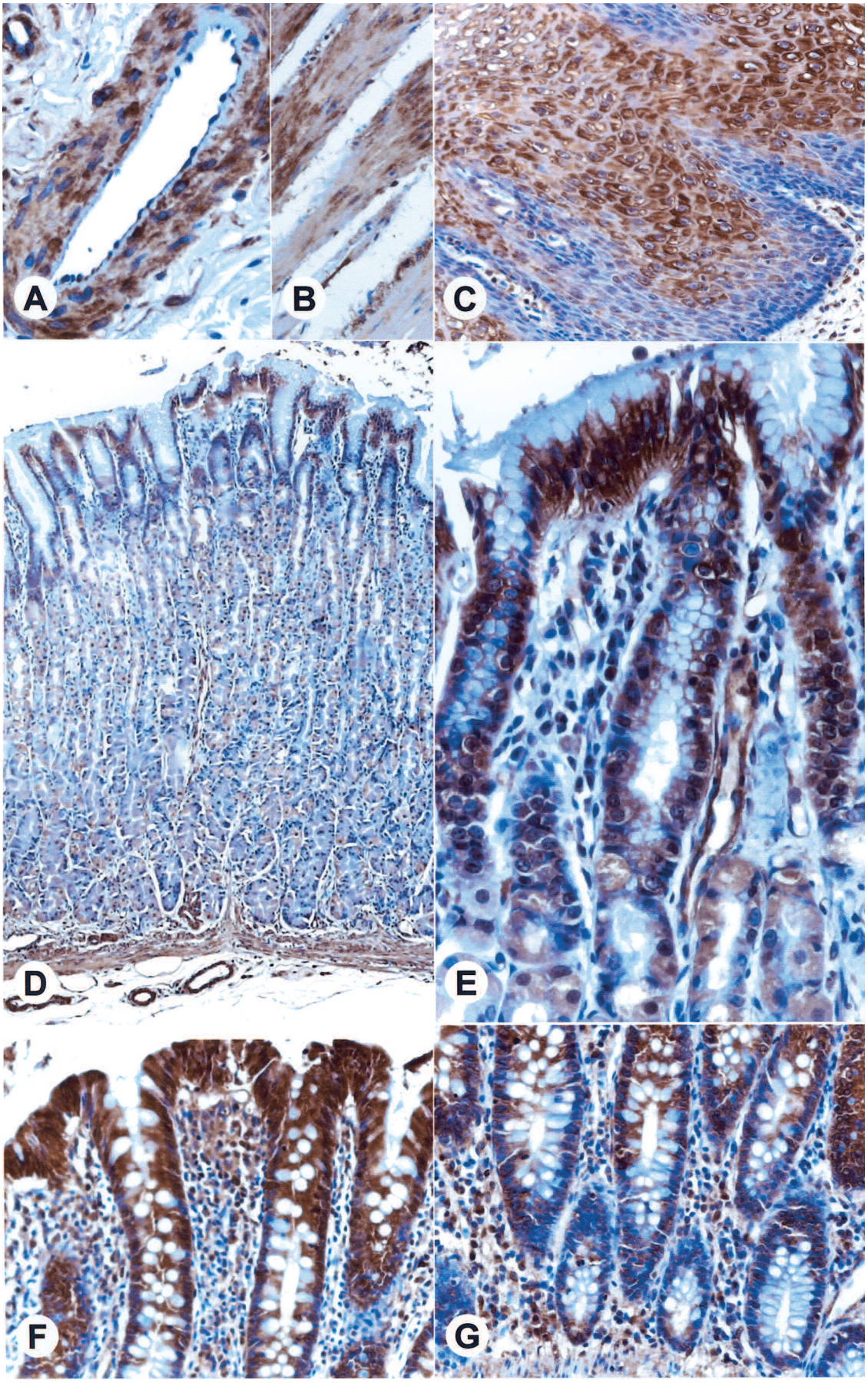

Fig. 5. MAC30 expression and localization in gastrointestinal tissues. Immunohistochemistry was performed as described in the Methods sections. **A.** MAC30 immunostaining in the endothelium and smooth muscles of a blood vessel and **(B)** the muscularis layer are shown. MAC30 immunoreactivity in the epithelium of the normal esophagus **(C),** normal stomach **(D, E**) and normal colon **(F, G)**. Original magnification: A-C, E-G, x 200; D, x 20

immunoreactivity (Fig. 5C). Furthermore, strong MAC30 immunoreactivity was present in the endothelial and smooth muscle cells of the blood vessels and in the muscularis layer of the esophagus (Fig. 5A,B). Normal gastric mucosal cells exhibited intense cytoplasmic MAC30 immunostaining which was more pronounced in the parietal cells (Fig. 5D,E). The gastric submucosa and muscularis exhibited MAC30 immunostaining with the same pattern and distribution as described for the normal esophagus. The normal colonic epithelium also exhibited strong cytoplasmic MAC30 immunostaining (Fig. 5F,G). Analysis of the respective cancerous samples of the esophagus, stomach and colon revealed strong MAC30 immunoreactivity in the cancer cells of these samples (Fig. 6).

Discussion

In the current study, the expression of MAC30 was analyzed by QRT-PCR, in situ hybridization and immunohistochemistry in the normal pancreas, CP and pancreatic cancer tissues. By QRT-PCR MAC30 mRNA levels were below the normal range in approximately half of the pancreatic cancer tissues and 1/8 of CP tissues. In contrast, approximately 1/3 of pancreatic cancer and CP tissue samples expressed MAC30 mRNA at increased levels compared to normal pancreatic tissues. This different expression pattern of MAC30 mRNA by QRT-PCR in the examined pancreatic tissues may be due to the fact that certain elements in pancreatic cancer and CP tissues strongly express MAC30, and the difference in the content of these elements in CP and cancer compared to normal pancreatic tissues may affect the expression analysis to a great extent. MAC30 was expressed at moderate to low levels in normal pancreatic acinar cells, and even lower levels were observed in most cancer cells. In contrast, MAC30 was strongly present in tubular complexes, which constitute

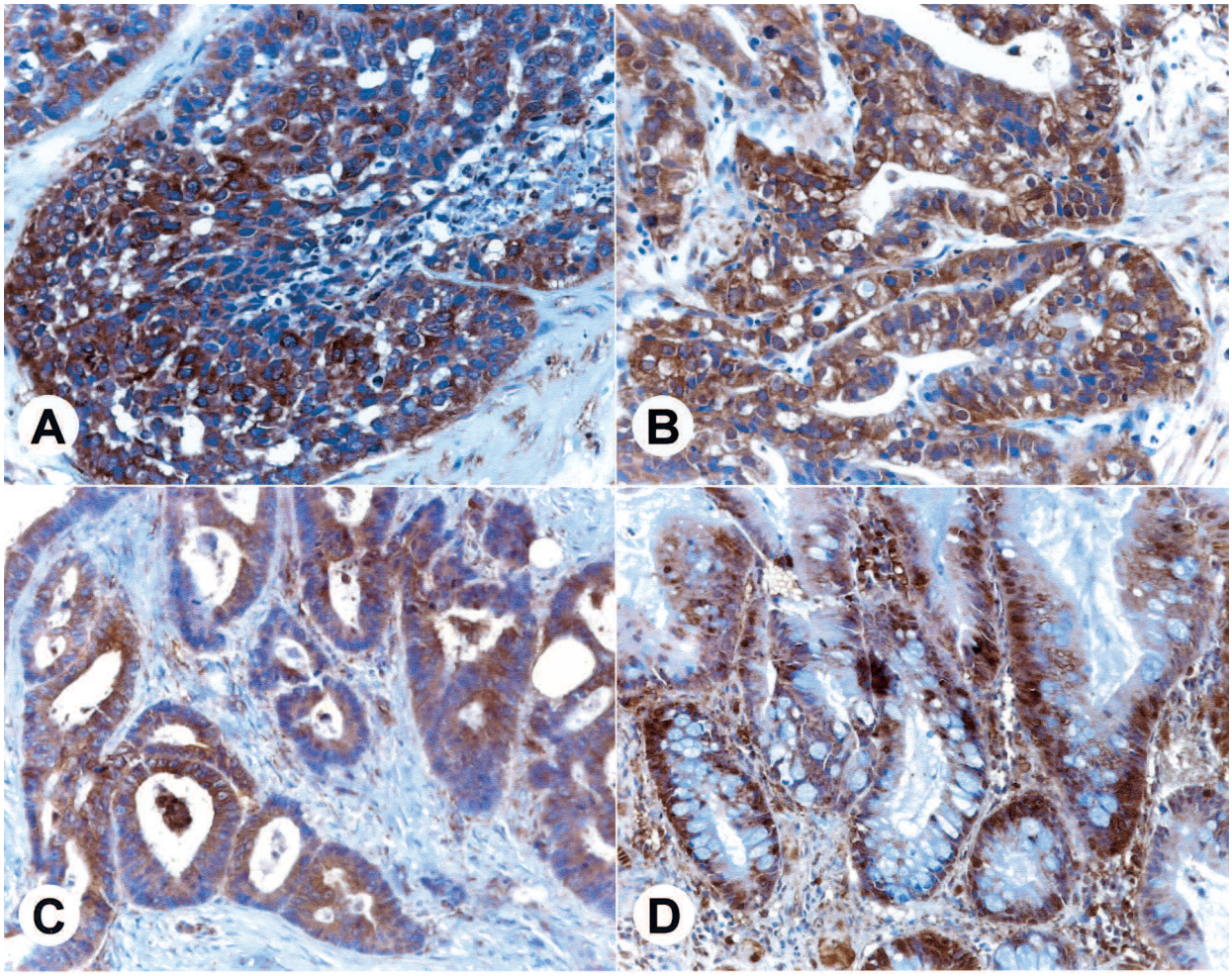

Fig. 6. MAC30 expression and localization in different gastrointestinal cancers. Immunohistochemistry was performed as described in the Methods sections. MAC30 immunolocalization in esophageal cancer **(A)**, stomach cancer **(B)** and colon cancer **(C, D)**. Original magnification x 200

characteristic morphological features of CP and CP-like lesions in pancreatic tumors. These tubular complexes together with the cancer cells are the most prominent difference between normal, CP and pancreatic cancer tissues, and they may be the reason behind the difference of MAC30 mRNA expression by QRT-PCR in these tissues. Tubular complexes are thought to evolve through trans-differentiation of acinar cells and to a lesser extent probably also of pancreatic islet cells (Bockman et al., 2003). This process does not require cell division and proliferation, both of which are key features of malignant transformation and cancer development (Bockman et al., 2003). The fact that MAC30 was observed in islets and acinar cells, markedly in tubular complexes and at low levels in pancreatic cancer cells suggests that it plays a direct or indirect role in the process of trans-differentiation of these cells. Since tubular complexes are considered potential preneoplastic lesions (Jimenez et al., 1999), the observed loss/reduction of MAC30 expression in pancreatic cancer suggests that this gene might act as a tumor suppressor in this malignancy. That MAC30 is involved in differentiation rather than proliferation is supported by various observations. Thus, MAC30 mRNA expression is down-regulated in human fibroblasts in response to serum (Iyer et al., 1999), which is known to provide growth factors to the cells which are mandatory for cell growth and proliferation. Moreover, ectopic expression of telomerase, which results in reduced requirements for endogenous mitogenes (Smith et al., 2003), leads to MAC30 mRNA down-regulation in human mammary epithelial cells (Smith et al., 2003). Furthermore, MAC30 is down-regulated by c-Jun N-terminal kinase antisense oligonucleotides (JNK2AS)in human PC3 prostate carcinoma cells (Potapova et al., 2002), in conjunction with growth suppression and induction of apoptosis in these cells (Potapova et al., 2002). These data together suggest that MAC30 is not involved in cell proliferation, but more likely in cellular differentiation, growth inhibition, or apoptosis. In support of this hypothesis, MAC30 is also a transcriptional target of the p53 tumor suppressor gene (Kannan et al., 2001).

In order to find out other possible regulators of MAC30 mRNA transcription, we examined the effects of TGF-ß1 on MAC30 transcription. TGF-ß1 significantly down-regulated MAC30 mRNA expression in two TGF-ß1-responsive pancreatic cancer cells. Inasmuch as most pancreatic cancers are resistant to TGF-, signaling due to Smad4 mutations (Hahn et al., 1996), Smad6/7 overexpression (Kleeff et al., 1999a,b), or reduced expression of TGF-, receptor I (Wagner et al., 1998), enhanced MAC30 expression in a subset of pancreatic cancers might be due to the loss of the repressive effects of TGF-ß on MAC30 transcription.

MAC30 expression is not only limited to the pancreas; strong MAC30 expression was also demonstrated in other gastrointestinal tissues. Thus, normal esophagus, stomach and colon tissue samples displayed MAC30 immunoreactivity predominantly in the mucosal cells. In contrast to pancreatic cancer tissues, the expression of MAC30 was strong in the cancer cells of stomach, esophagus and colon tumors.

In conclusion, MAC30 is expressed in a wide variety of human tissues, and shows a significant difference of expression between normal and cancerous tissues derived from the pancreas, colon, breast and kidney. MAC30 may be involved in the pathophysiological processes of tubular complex formation (i.e., acinar and islet cell trans-differentiation) in CP and CP-like lesions in pancreatic cancer. The low levels of MAC30 observed in pancreatic cancer are in contrast to the levels observed in stomach, esophagus and colon cancer, and suggest a distinct role of this gene in the pathogenesis of these tumors.

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